Microbiologist should use an optimal and clear presentation for susceptibility results, cumulative reporting, tracing of epidemiological strains and adequate comments to optimally inform clinicians.

S27 – New technologies and advances in the diagnosis...

WeS21 Alternatives to microscopic diagnosis of malaria

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Microscopy of Giemsa-stained thick and thin films remains the current reference method to diagnose malaria. However, it is time-consuming, requires expertise in microscopy and maintenance of equipment. In developing nations scarce resources lead to inadequate diagnostic procedures, while in affluent countries poor familiarity with malaria may cause misdiagnosis. Several alternative tests have been developed recently. Microscopy with fluorescent stains, including centrifugation (QBC®), show sensitivities and specificities similar to conventional microscopy, although much quicker to perform. However, they need special equipment and training, especially in the differentiation of species. Dipstick antigen-detection of parasite Histidine-Rich-Protein II (HRP II) (Parasight®-F, ICT Malaria®) Lactate dehydrogenase (OptiMAL®) or HRPII/Aldolase (ICT Malaquick Pf/Pv®) are also quick to perform and do not need any equipment. These tests show sensitivities and specificities comparable to microscopy, although at lower parasitaemias sensitivities may decrease significantly, and they detect only *P. falciparum* (ParasightF®, ICT Malaria®) or *P. falciparum* and *vivax* (OptiMAL®, ICT Malaquick Pf/Pv®). These dipstick-tests may be useful in remote areas without laboratory access or in laboratories as back-up to microscopy, e.g. for non-experts during on-calls. Polymerase-chain-reaction assays are more sensitive and specific than microscopy, however, they are of limited value in the diagnosis of acute malaria. Automated blood cell analysers (Cell-Dyn 3500®) are a novel way to diagnose malaria during routine use for Full-Blood-Counts and may have the potential to detect malaria cases, even in the absence of clinical suspicion.

WeS22 Visceral leishmaniasis: New diagnostic approaches

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The standard technique for the diagnosis of first episodes and relapses of visceral leishmaniasis (VL) is the bone marrow aspirate microscopy and culture, which results in > 95% sensitivity. However, this is an invasive procedure which requires experienced microscopists and the availability of freshly prepared culture media. New diagnostic approaches have been proposed which, however, must be treated differently in the two cohorts of VL patients, i.e. HIV-negative and HIV-infected individuals.

VL in HIV-negative individuals - Antileishmanial antibodies are detected in 99–100% of these patients by a number of immunological tests which use *Leishmania* crude antigens. Recently, test specificity was greatly increased by the use of recombinant antigens. Serology should represent the first and, probably, the sole diagnostic approach in immunocompetent individuals. Furthermore, serial titration of specific antibodies at 2–3 months intervals has high prognostic utility in the follow-up of drug treated patients.

VL in HIV-infected individuals - Less than 60–70% of these patients have detectable antileishmanial antibodies; however, 70–75% have parasites in peripheral blood. Sensitive techniques have been developed to detect microscopically *Leishmania* on leukocytoconcentrates, or to amplify leishmanial DNA (PCR) from buffy-coat or blood-spot filter paper samples. The use of these noninvasive techniques is of great value in the patient's follow-up.

S28 – Pathogenesis for gram-positive sepsis and shock

WeS24 Advances in the diagnosis of Toxoplasma infection

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Toxoplasma infections are wide spread and occur all over Europe. The infection is usually benign, but can induce severe complications if it occurs in a developing fetus or an immunocompromised patient.

Exposure to infection varies regionally. From 30–90% of women are non-immune and susceptible to infection. 0.5–5 women per 1000 acquire the infection during pregnancy with an over all fetal transmission rate of 30%.

The diagnosis of T. infection depends either on identification of parasites in tissue/body fluids or on detection of specific antibodies. In pregnancy the diagnostic challenge lies in differentiating between *primary* maternal infection which may cause fetal infection and *past latent* infection which is without importance. In the fetus and neonate the challenge is to identify infected cases at an early stage, while in patients with chorioretinitis or AIDS it is important to decide if Toxoplasma is involved.

An arsenal of diagnostic assays is available for detection of Toxoplasma specific antibodies. The *EIA method is* mostly used both for IgG, IgM and IgA detection. The direct agglutination is suitable for screening, while recently the *IgG avidity* test has proven useful in determining the time of infection.

The PCR method has found its place in diagnosis of fetal infection where the presence of Toxoplasma DNA in amniotic fluid indicates maternal fetal transmission.

In the HIV patient the antibody titers are low or undetectable. A definite diagnosis has to rely upon antigen detection. In ocular toxoplasmosis demonstration of antibody production in aqueous humor may be helpful.

WeS25 Contact phase activation and bacterial virulence

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A starting point for our studies was the observation that high-molecular-mass kininogen (H-kininogen) binds to the surface of several species of pathogenic bacteria. H-kininogen is a multifunctional plasma protein which together with the other components of the contact phase system, factor XI and factor XII of the blood coagulation system and plasma prekallikrein, participates in molecular events leading to the activation of the intrinsic pathway of blood coagulation, and to the release of bradykinin (BK) from H-kininogen. BK is a nonapeptide and a primary mediator of inflammation, which through the induction of secondary mediators such as prostaglandins and nitric oxide, induces pain, increased vascular permeability and vasodilatation. It was demonstrated that interactions between contact phase factors and bacterial surface proteins result in the release of BK at the site of infection. Furthermore, an extracellular cysteine proteinase produced by *Streptococcus pyogenes* induces a rapid breakdown of H-kininogen and massive BK release. Finally, absorption of contact phase proteins and fibrinogen by bacterial surface proteins causes a hypocoagulatory state. These molecular mechanisms may contribute to the bleeding disorders and the hypovolemic hypotension seen in patients with severe infections disease.

WeS26 The role of superantigens and bacterial DNA during Lethal Shock

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Endotoxin (LPS) has been defined to play a major role during pathogenesis of infection. Gram-positive bacteria do not contain LPS yet induce strong responses of innate immune cells. At least two products or components of gram-positive bacteria have been defined with similar effects. Once, superantigens like the staphylococcal enterotoxin B (SEB) activate oligoclonally T-lymphocytes. This activation results in a burst of cytokine secretion that can induce a lethal cytokine syndrome. Moreover, SEB induced interferon-γ (IFN-γ) modulates the status of macrophages and thus enhances their response to LPS. Second, bacterial DNA derived from gram-positive or gram-negative bacteria has been shown to be a potent activator of innate immune cells. DNA mediated activation is DNA sequence dependent and relies on the abundance of free CpG-dinucleotides which are less frequent and methylated in mammalian DNA (CG-suppression). Recent studies have shown, that even synthetic oligonucleotides (ODN) displaying a certain DNA motifs activate efficiently innate immune cells like macrophages or dendritic cells. Bacterial DNA and CpG-ODN induce production of large amounts of pro-inflammatory cytokines which can induce lethal shock. However, CpG-DNA also can be used to modulate ongoing immune responses. This effect of CpG-DNA can be used to develop new adjuvants which preferentially induce Th1-immune responses.